SENT VIA FACSIMILE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	: Richard Ian Christopherson *et al.*	Docket	: 650061.401C1
Serial No	: 09/888,959	Group Art Unit	: 1642
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For	: Assay to detect a binding partner		

Commissioner of
Patent and Trademarks
Washington, D.C. 20231

DECLARATION PURSUANT TO 37 C.F.R. §1.132

I, Professor Richard Ian Christopherson, hereby declare as follows:

1. I am currently Professor of Biochemistry in the School of Molecular and Microbial Biosciences at the University of Sydney, Maze Crescent, Sydney, New South Wales, 2006, Australia.

2. I have pursued research in the area of biochemistry, molecular and cellular biology and have published extensively in these fields. A list of appointments I have held during my career together with my publications are included in my Curriculum Vitae which is attached hereto as Exhibit **RIC-1**.

3. I am an inventor of subject matter (hereinafter referred to as the "INVENTION") described and claimed in United States Patent Application Serial No. 09/888,959 filed on 25 June, 2001 (hereinafter referred to as the "APPLICATION"). The APPLICATION contains claims directed *inter alia* to a method for determining a unique profile of molecules, wherein the profile is indicative of a particular type of leukemia.

4. Leukemia is a debilitating disease which causes stress for both the patient and family. Further, the financial costs of leukemia can be great, not only for the individual but also for society. The key to the successful treatment of leukemia is an accurate and early diagnosis which allows for effective therapeutic intervention.

5. Leukemia is a malignant neoplasm of blood-forming tissues, characterized by abnormal proliferation of leukocytes. Leukemias are classified as either lymphoid or myeloid, depending on the type of leukocyte affected. In addition, leukemias are classified as either acute, referring to a rapidly progressing disease that involves immature leukocytes, or chronic, referring to a slower proliferation involving more mature white cells.

In acute leukemias, immature non-functional leukocytes called blast cells proliferate. The myeloid leukemias affect white blood cells (myelocytes) giving rise to basophils, neutrophils, eosinophils, monocytes, megakaryocytes and erythrocytes which have a variety of roles in immune defence, clotting and oxygen transport. Mutations in precursors of these cells may give rise to chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) also called acute non-lymphocytic leukemia (ANLL). The lymphocytic leukemias arise from mutations in the precursors of B- and T-lymphocytes. They include acute lymphocytic leukemia (ALL); chronic lymphocytic leukemia (CLL); and hairy cell leukemia (HCL), a chronic leukemia named for the cells' tiny hair-like projections. The lymphocytic leukemias are sometimes referred to as B or T cell leukemias depending upon whether they arise from cells in the pathways of differentiation (lineages) leading to antibody-producing B cells (HCL, CLL, and some cases of ALL) or T lymphocytes involved in cell-mediated immunity (some cases of ALL). While the major categories of leukemia are CML, AML, CLL and ALL, there are many other types of cancer which involve blood cells (leukemias, lymphomas and myelomas) which include, promyelocytic leukemia, multiple myeloma, non-Hodgkin's lymphoma, plasmacytic tumors, natural killer large granular lymphocytic (NK LGL) leukemia, T large granular lymphocytic leukemia, mantle cell lymphoma, lymphomatous polyposis, small lymphocytic lymphoma, follicular

lymphoma, Burkitt's lymphoma, peripheral T cell lymphoma, marginal zone lymphoma, MALToma and splenic lymphoma with villous lymphocytes.

Traditionally, when diagnosing a specific type of leukemia, the first step is to distinguish between lymphoid and myeloid sub-types by examining the morphology of the cells. The next step is to help rule-out the chance that an increased number of leukocytes is due to an infection. This is known as the "leukemoid reaction". For this, a test called Lymphocyte (or Neutrophil) Acid Phosphatase ("LAP") is done on the cells and the LAP score calculated. High LAP scores indicate infection.

Once it has been determined that a subject is suffering from a leukemia, it is then important to determine the type of leukemia for prognosis and appropriate treatment. This typically requires that a patient's sample is immunophenotyped; the expression pattern of cell surface molecules is determined on leukemia cells in the sample wherein the particular pattern is indicative and characteristic of a specific leukemia sub-type. Immunophenotyping provides information on the stage of differentiation of the cell when it became leukemic. Until the advent of the INVENTION, only limited immunophenotyping was possible with just a few CD antigens being detected by flow cytometry. Due to this limitation, the surface molecule information needed to be taken with morphology, cytochemistry and cytogenetics to provide a diagnosis according to the FAB (French-American-British) or WHO systems.

Different types of leukemias can be distinguished by the repertoire of surface molecules present on the leukemic cell. However, many leukemias share common CD antigens. For example, hairy cell leukemias are $CD19^+$, $CD5^-$, $CD10^-$, $CD11c^+$, $CD25^+$ and $CD103^+$, whereas marginal zone lymphomas are $CD19^+$, $CD5^-$, $CD10^-$, $CD11c^+$ and $CD25^+$. As such, in order to accurately distinguish between just these two types of cancer using cell surface markers, it would be necessary to examine the expression of as many CD antigens as possible. The INVENTION makes it possible to distinguish between all of the common types of leukemias from an extensive immunophenotype using a large panel of antibodies specific for a range of surface molecules.

Traditional methods of phenotyping include ELISA and flow cytometric analysis. However, there are severe limitations in using such methods to obtain an extensive immunophenotype for diagnosis of a specific leukemia. For instance, flow cytometry analyses biological material by detection of the light-absorbing or fluorescing properties of cells wherein an absorbance or fluorescence profile of the sample is produced. This profile is based on the staining of a population of cells using fluorescently-labeled antibodies against surface markers. Light is emitted by each fluorochrome at a characteristic wavelength. This analysis is limited by the number of different flurochromes available which emit light at distinguishable wavelengths and by the number of lasers emitting light at different wavelengths to excite these flurochromes. Currently, the most advanced research flow cytometers allow the evaluation of only a few different surface markers and antibodies specific to CD antigens and coupled to distinguishable fluorophores are not available commercially and would need to be synthesized and purified on-site. Therefore, to analyze a panel of, for example, 70 surface markers would require analysis of more than 8 experimental tubes of cells plus a large number of controls. Accordingly, such flow cytometric assays are not suitable for routine diagnosis of leukemias from patients based solely upon an extensive immunophenotype due to excessive time and cost.

6. The INVENTION arose from this clear need to develop a more rapid, robust and cost effective method to diagnose leukemias as early as possible so that the appropriate treatment could be provided.

7. The INVENTION relates to a method of determining the specific type of leukemia using a one step analysis based solely on a visual pattern of markers captured concurrently by binding partners on a solid phase, rather than analysis using assays which measure only a small number of binding events (flow cytometry). The assay of the present invention generates patterns based on the presence or absence of particular markers from leukemia cells in a single patient sample. It is the collective visual pattern of binding events on a solid phase, i.e. the pattern of presence or absence of

these discriminatory markers, which is indicative of a specific type of leukemia in a patient.

The INVENTION contemplates the use of microarray device with immobilized binding partners of discriminatory markers (antibodies) which have been associated with different types of leukemia. The power of this methodology can be seen in the Venn diagrams ***attached*** as exhibits **RIC-2 to RIC-5**. These exhibits demonstrate how the specific patterns of binding associated with a particular leukemia can be so complex. For example, a patient whose sample is positive for the markers CD1a, CD56, CD5, CD2, CD57, CD38, CD4, CD103, CD44, CD95, CD10, CD45, CD95, TCR-β, TCR-γ, CD7, CD11b, CD11a, CD11c, CD49d, CD102, CD122, CD28, CD16, CD25, CD52, CD126, CD49e, CD128, CD29, CD62L, CD130, CD71, CD134, CD43, CD80, CD60 and CD154, but negative for CD19, CD20, CD77, CD21, CD79a, CD22, CD79b, CD37, CD9, CD23, CD24, CD31, CD54, CD32, CD86, CD40, HLA-DR, FMC7, CD120a, CD62P, CD13, CD14, CD41, CD64, CD88, CD235a, CD42a, CD33, CD61 and CD66c would be diagnosed as having a T-cell associated leukemia (as seen in exhibit **RIC-2**). Furthermore, using the same markers, one can also accurately diagnose B cell leukemias, myeloid leukemias and stem cell leukemias. Hence, using a panel of 63 markers combined in a single assay, one can accurately diagnose the sub-type of leukemia in a 5-mL blood sample from a patient, allowing the specific tailoring of their treatment. Such an analysis, even when using an assay such as flow cytometry would require the running at least 8 experimental tubes plus controls, a labor intensive and expensive process.

Using this assay, we can rapidly identify a particular leukemia far more efficiently and accurately than could have been achieved by conventional analysis.

8. The commercial potential of the INVENTION is evident by a substantial licensing arrangement entered into between the University of Sydney and a medical diagnostic company. This company has recognized that the INVENTION provides a solution to the immediate need to improve and expedite diagnosis of leukemias.

The undersigned declares further that all statements made herein are of his own knowledge, are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Date: 10 February 2004 R. I. Christopherson

Professor Richard Ian Christopherson

Executed in the Presence of

ARTHUR DAVID CONIGRAVE

Name

Conigrave

Signature

DEPUTY HEAD OF SCHOOL

Position

10 Feb 2004

Date